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Kidney Function and Sulfate Uptake and Loss in the Freshwater Bivalve *Toxolasma texasensis*

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Abstract. *Toxolasma texasensis* acclimated to an artificial pondwater (PW) maintained a concentration of SO_4 in the blood of about 1–2 mmol l^{-1}. The anion transport inhibitor DIDS (5, 5′-diisothiocyanatostilbene 2, 2′-disulfonic acid) reduced the uptake of $^{35}SO_4$ from the bathing medium by 54%. The clearance of polyethylene glycol (PEG) injected into the blood of *T. texasensis* ranged between 0.8 and 1.3 ml g^{-1} dry tissue h^{-1}, and provided an estimate of renal filtration in PW-acclimated animals. The clearance of radioactive $^{35}SO_4$ simultaneously injected into the same animal was about 16% of the PEG clearance, suggesting that sulfate was being reabsorbed by the kidney. Para-aminohippuric acid was cleared about 4.6 times faster than PEG, indicating that this organic acid was subjected to secretion in addition to filtration. When the normal osmotic gradient was abolished by acclimating *T. texasensis* to 10% seawater (SW), the PEG clearance decreased to 0.17 ml g^{-1} dry tissue h^{-1}. Sulfate clearance in animals acclimated to PW or 10% SW was the same. However, in mussels acclimated to 10% SW, the calculated amount of SO_4 reabsorbed was significantly reduced relative to mussels acclimated to PW. *T. texasensis* conserved SO_4 when acclimated to PW, and reduced reabsorption when acclimated to the sulfate-rich 10% SW. When mussels acclimated to 10% SW were returned to PW, there was a transient increase in sulfate clearance during the first 8 h because filtration exceeded reabsorption.

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Abbreviations: DIDS, 5, 5′-diisothiocyanatostilbene 2, 2′-disulfonic acid; PAH, para-aminohippuric acid; PEG, polyethylene glycol; PW, artificial pondwater; SW, artificial seawater.

Introduction

The characteristics of solute (K, La, Na, sucrose, mannitol) penetration through the epithelia of the unionid *Toxolasma texasensis* are intermediate to those of other freshwater bivalves (Dietz and Byrne, 1990; Dietz *et al.*, 1995; Wilcox and Dietz, 1995; Byrne and Dietz, 1997; Zheng and Dietz, 1998b; Dietz and Byrne, 1999). Previous studies indicated that the passive movements of solutes and water across the epithelia of *T. texasensis* were relatively slower that those of the dreissenid *Dreissena polymorpha* (Scheide and Dietz, 1986; Dietz and Byrne, 1997, 1999). To maintain ionic homeostasis, an animal must be able to accumulate and retain solutes; then to preserve water balance, it must excrete a volume of water equivalent to that taken up osmotically. Kidney filtration can be estimated by measuring the clearance of marker solutes from the blood, and it is a useful index from which kidney function and osmotic water movement can be monitored (Potts, 1954b; Murphy and Dietz, 1976; Hevert, 1984; Kirschner, 1991; Dietz and Byrne, 1997, 1999).

Unionid bivalves accumulate sulfate at the rather slow rate of 0.04 μmol g^{-1} dry tissue h^{-1} (Dietz, 1978). Thus, sulfate is a relatively nonpenetrating anion, and therefore it has been used for short-term studies of independent ion transport (Krogh, 1939; Scheide and Dietz, 1982; Byrne and Dietz, 1997; Zheng and Dietz, 1998a; Dietz and Byrne, 1999). However, sulfate is present in millimolar concentrations in the blood of freshwater mussels (Potts, 1954a; Dietz and Byrne, 1999) and is a component of various organic molecules (*e.g.*, amino acids, mucopolysaccharides) found in molluscs (Eriksson *et al.*, 1984; Kornprobst *et al.*, 1998).

Sulfate balance was studied recently in *D. polymorpha*, which has the highest epithelial solute permeability of any freshwater bivalve tested (Dietz and Byrne, 1999). In the present study, kidney function was examined in a unionid

bivalve, *Toxolasma texasensis*. The unionids have a longer freshwater ancestry (Triassic) than the dreissenids, which invaded freshwater in the Pleistocene (Haas, 1969). Sulfate uptake and the characteristics of the renal clearance and conservation of $^{35}SO_4$ that was injected into the body fluids were studied.

Materials and Methods

Animal acclimation

Toxolasma (=*Carunculina*) *texasensis* was collected from ponds near Baton Rouge, Louisiana. The animals were stored, unfed for at least 1 week before use, in aerated artificial pondwater (PW) at 22° ± 2°C. The pondwater composition (in millimoles per liter) was 0.5 NaCl, 0.4 $CaCl_2$, 0.2 $NaHCO_3$, 0.2 $MgSO_4$, 0.05 KCl (Dietz *et al.*, 1994). Some mussels were acclimated to an artificial seawater (SW) that was diluted with PW to be about 10% SW (~106 mosm kg^{-1}) for more than 13 d before use. In 10% SW the mussels become isosmotic, and the osmotic gradient is minimal. The stock SW composition (in millimoles per liter) was 449.1 NaCl, 27.5 $MgSO_4$, 24.4 $MgCl_2$, 9.9 $CaCl_2$, 6.6 KCl, 2.4 $KHCO_3$, 0.8 KBr, 0.4 H_3BO_3, 1076 mosm (salinity = 35‰) (Chambers and De Armendi, 1979).

Solute analyses

Samples of blood (200–300 μl) were obtained from mussels by pericardial puncture and centrifuged for 3 min at 8000 × *g* before analysis (Fyhn and Costlow, 1975). The osmolality of blood or bathing medium was determined on undiluted samples by freezing point depression. Sodium and potassium concentrations were determined by emission flame photometry. Calcium and magnesium concentrations were determined by atomic absorption spectroscopy from samples diluted with La_2O_3/HCl. Chloride concentration was determined by electrometric titration.

Sulfate was determined by a turbidimetric method that formed a precipitate with barium in a total volume of 0.8 ml (Dietz and Byrne, 1999). A 50-μl sample of blood or bathing medium was added to 450 μl deionized water. To each sample, 100 μl of 4.1 mol l^{-1} NaCl in 0.2 mol l^{-1} HCl was added and vortexed. A 100-μl aliquot of glycerol, ethanol, dibutyl phosphate (2:1:0.15 by volume) was added and vortexed. The SO_4 was precipitated as $BaSO_4$ crystals by the addition of 100 μl of 1 mol l^{-1} $BaCl_2$ and immediately sonicated for 10 s to form crystals of a reproducible size (Dietz and Byrne, 1999). Turbidity was measured spectrophotometrically at 400 nm and was compared to a standard curve that was linear up to 1 mmol l^{-1} Na_2SO_4.

Sulfate uptake

Sulfate uptake was measured by the appearance of the radiolabeled SO_4 in the blood (Dietz and Byrne, 1999). Each animal was placed in a separate container and used once. The bathing medium was PW (0.2 mmol l^{-1} SO_4), and trace amounts of $^{35}SO_4$ were added to give a specific activity of 100,000 dpm μmol^{-1}. After 3 h, bath samples were collected, and the radioactivity was measured using a xylene/Triton X-114 liquid scintillation cocktail (Wiegman *et al.*, 1975) and a Beckman LS6000 counter. After the samples were collected, each mussel was removed, blotted dry, and weighed; a blood sample was then taken and processed as described above. The tissue was removed from the shell, and the soft tissue was dried overnight (90°C) to determine dry mass. The amount of $^{35}SO_4$ that accumulated in the mussel blood during the exposure to the isotope was used to calculate the uptake of sulfate (nanomoles per milliliter) in the blood by dividing the amount of radioactivity in a known volume of blood (dpm per milliliter) by the specific activity of the bathing medium (dpm per nanomole). In some studies, SO_4 uptake was measured for 3 h from PW containing 0.5 mmol l^{-1} of the anion transport inhibitor 5, 5′-diisothiocyanatostilbene 2, 2′-disulfonic acid (DIDS) adjusted to pH 7.3 with Tris. The uptake studies were performed under indirect illumination to minimize photodegradation of DIDS.

Solute clearance

Radioactive tracers [^{3}H-polyethylene glycol (PEG, 4 kDa), and Na_2 $^{35}SO_4$] dissolved in deionized water were injected [10 μl, 1 μCi of each isotope (1 Ci = 37 GBq)] into the foot muscle of each specimen, and the animal was returned to the appropriate acclimation medium for 3 h to allow isotope equilibration. The clearance of the radioisotope from the body fluids of *T. texasensis* was determined using the procedures of Dietz and Byrne (1997, 1999). In brief, after the 3-h equilibration, the animals were rinsed three times to remove adsorbed isotope and transferred to separate containers with 30 ml of the appropriate experimental bathing medium. The mussels resumed siphoning within 10–20 min, bath samples were collected at times 0 and 1 h, and the radioactivity was determined by double-label counting procedures, as needed, using a programmable Beckman LS6000 scintillation counter. After the final sample of bathing medium was taken, each mussel was removed, blotted dry, and weighed; a blood sample was then collected. The tissue was removed from the shell, and the soft tissue was dried overnight at 90°C and reweighed.

The clearance of the isotope was calculated from the total amount of radioactivity that accumulated in the bathing medium during the 1-h interval (dpm per hour) divided by the isotope radioactivity in the blood (dpm per milliliter) at the end of the clearance study (Murphy and Dietz, 1976). The clearance of solute from the blood was expressed as milliliters of blood per gram of dry tissue per hour. Because of the potential damage due to pericardial sampling, blood

was collected only at the end of the experiment. Calculated data assumed that the specific activity of the blood remained constant during the 1-h clearance measurement. The specific activity of the blood probably decreased exponentially during the experiment, and clearance may be underestimated by 20%–24% (Murphy and Dietz, 1976; Dietz and Byrne, 1997).

Urine samples were not collected from the bivalves; thus the urine volume and urine:blood ratio of radioactive tracer were not determined directly. The method used for calculating clearance would determine the equivalent volume of blood that would have to be cleared of the tracer by all routes (kidney, epithelial, digestive tract), but our previous studies indicated that most of the loss is renal (Dietz and Byrne, 1997) and that contamination of the blood with bathing medium was rare and minimal (Dietz *et al.*, 1997).

The PEG marker was considered to represent the amount of material filtered by the kidney in bivalves (Dietz and Byrne, 1997, 1999). Thus, clearance values of other solutes(x) were compared with PEG clearance for each specimen to distinguish filtration (equal clearance values), reabsorption [clearance(x) < PEG], or secretion [clearance(x) > PEG]. Sulfate clearance was calculated by the method described above for PEG. The sulfate concentration in the blood of each specimen was measured and converted into the amount of sulfate filtered (micromoles of sulfate per gram of dry tissue per hour) into the kidney by multiplying the PEG clearance (milliliters of blood cleared of PEG per gram of dry tissue per hour) by the blood sulfate concentration (micromoles of sulfate per milliliter of blood). Knowing the specific activity (dpm μmol^{-1}) of $^{35}SO_4$ in the blood, the quantity of $^{35}SO_4$ excreted (dpm per gram of dry tissue per hour) was converted into the total quantity of sulfate eliminated (micromoles of sulfate per gram of dry tissue per hour) for each specimen, and this value represented sulfate excretion. The sulfate reabsorption was calculated as the difference between the filtered and excreted sulfate values for each animal.

Clearance studies were performed on mussels acclimated either to PW or to 10% SW: animals in PW were hyperosmotic to the bathing medium; those in 10% SW were isosmotic. Animals acclimated to 10% SW were transferred to PW for 1, 4, 8, 24, 48, or 72 h to observe the changes in renal clearance when they experienced an increased osmotic gradient. Clearance was measured for 1 h, ending at each time interval specified, and the amounts of sulfate filtered, excreted, and reabsorbed were calculated.

Para-aminohippuric acid (PAH, 194 Da) was injected into mussels together with PEG (15 μl, 1 μCi) to compare clearance values. The method used was similar to that described above for the double-label ^{3}H-PEG and $^{35}SO_4$ studies. Both ^{3}H- and ^{14}C-label for both PEG and PAH, and identical results were obtained. The clearance of PAH was rapid, thus the equilibration time was shortened from 3 h to 1 h.

Table 1

Blood solute concentration in pondwater-acclimated Toxolasma texasensis *with or without exposure to 0.5 mmol l^{-1} DIDS for 3 h*

Solute	Control	Treated
Total solute, mosm kg^{-1}	40 ± 2	39 ± 2
Na, mmol l^{-1}	19.4 ± 1.3	19.9 ± 1.2
K, mmol l^{-1}	0.4 ± 0.0	0.5 ± 0.1
Ca, mmol l^{-1}	3.5 ± 0.2	3.4 ± 0.2
Mg, mmol l^{-1}	0.3 ± 0.0	0.4 ± 0.0
Cl, mmol l^{-1}	12.3 ± 0.6	12.5 ± 0.8
SO_4, mmol l^{-1}	1.8 ± 0.2	1.7 ± 0.1
$^{35}SO_4$, nmol ml^{-1}	13 ± 1	6 ± 2*

Data are mean ±1 standard error, $n = 5$, * $P < 0.05$.

Statistical analyses

All data are expressed as the mean ± 1 standard error (SE). An animal was used once, and *n* indicates the number of animals in each treatment group. Data were analyzed for differences between treatment groups by performing a one-way analysis of variance (ANOVA). When the ANOVA was significant, the Fisher's protected least significant difference method was used to determine differences between specific means ($P < 0.05$).

Results

Solute balance and SO_4 uptake

Toxolasma texasensis is a hyper-regulator in freshwater, and the solute concentrations measured in the blood were maintained at higher levels than those in the PW bathing medium (Table 1). Sulfate had the lowest concentration of any anion in the blood (1.7–1.8 mmol l^{-1}), but this concentration was about 8 times higher than in the medium (0.2 mmol l^{-1}). Thus, SO_4 is concentrated to the same level as Cl (Table 1).

The SO_4 influx is slow in unionids (Dietz, 1978); thus we did not measure the unidirectional influx of SO_4 by the disappearance of isotope from the bathing medium. Less than 1000 dpm ml^{-1} of $^{35}SO_4$ would disappear from the bath after several hours, and compared to the 20,000 dpm ml^{-1} (30 ml bath volume) present in the bathing medium, this difference could not be distinguished with liquid scintillation counting techniques. The amount of $^{35}SO_4$ that accumulated in the blood over the incubation interval was small, but significantly greater than zero. The uptake of $^{35}SO_4$ was reduced 54% (significant at $P < 0.05$) by exposure to 0.5 mmol l^{-1} DIDS, but none of the other solutes measured in the blood were affected (Table 1).

Table 2

Volume of blood cleared of polyethylene glycol (PEG) and $^{35}SO_4$, and calculated sulfate processing by the kidney of Toxolasma texasensis *acclimated to pondwater (PW), 10% seawater (SW), or when returned to PW for various periods*

		Clearance, ml g^{-1} dry tissue h^{-1}		Sulfate, μmol g^{-1} dry tissue h^{-1}		
Treatment	*n*	PEG	Sulfate	Filtered	Reabsorbed	Excreted
10% SW	10	0.17 ± 0.05a	0.09 ± 0.02a	0.48 ± 0.14a	0.23 ± 0.08a	0.25 ± 0.07ab
1 h PW	5	0.57 ± 0.17b	0.21 ± 0.05a	1.34 ± 0.54b	0.84 ± 0.38ab	0.51 ± 0.18b
4 h PW	7	1.24 ± 0.12c	0.76 ± 0.13b	2.97 ± 0.37c	1.17 ± 0.32b	1.80 ± 0.31d
8 h PW	5	1.32 ± 0.18c	0.77 ± 0.15b	1.77 ± 0.25b	0.81 ± 0.25ab	0.97 ± 0.08c
24 h PW	5	1.25 ± 0.15c	0.09 ± 0.02a	1.66 ± 0.11b	1.54 ± 0.10bc	0.12 ± 0.02ab
48 h PW	5	1.32 ± 0.19c	0.10 ± 0.04a	1.74 ± 0.20b	1.62 ± 0.21bc	0.11 ± 0.03ab
72 h PW	5	0.93 ± 0.15bc	0.03 ± 0.00a	2.03 ± 0.39b	1.97 ± 0.38c	0.06 ± 0.01a
PW	11	0.76 ± 0.11b	0.12 ± 0.02a	1.33 ± 0.24b	1.17 ± 0.24b	0.17 ± 0.02ab

Data are expressed as mean ±1 standard error. Values within a column that have different letters are significantly different using Fisher's protected least significant difference method ($P < 0.05$).

Solute clearance

We previously reported values for clearance of radioactive inulin from the blood of *Ligumia subrostrata* and *T. texasensis* (Murphy and Dietz, 1976; Scheide and Dietz, 1986); in this study similar results were obtained using PEG: 0.77 ± 0.04 ml g^{-1} dry tissue h^{-1} ($n = 6$). However, the clearance of $^{35}SO_4$ administered simultaneously (0.09 ± 0.03 ml g^{-1} dry tissue h^{-1}) was 12% of the clearance of ^{3}H-PEG, suggesting that filtered SO_4 was being reabsorbed by the renal tissue.

The osmotic uptake of water should be high in PW-acclimated *T. texasensis*, but low in 10% SW-acclimated animals. Thus, filtration measured by the clearance of PEG should be at a relatively high rate in the former and lower in the latter acclimation medium. The clearances from animals that were doubly labeled with $^{35}SO_4$ and ^{3}H-PEG were measured from animals acclimated to either PW or 10% SW (Table 2). The animals in 10% SW were isosmotic with the medium (Table 3) and would have experienced a lower osmotic uptake of water and therefore reduced filtration, as reflected in the clearance of PEG. The sulfate clearance was about 16% of the PEG clearance in the PW-acclimated animals. However, the mussels acclimated to 10% SW had similar clearances ($P > 0.1$) for both $^{35}SO_4$ and ^{3}H-PEG. The SO_4 clearance in 10% SW-acclimated animals appeared to be unchanged relative to the PW-acclimated mussels. However, because the clearances of SO_4 and PEG were similar, these data suggest that 10% SW-acclimated animals had reduced their reabsorption of SO_4 (Table 2). The SO_4 concentration in the blood of *T. texasensis* acclimated to 10% SW was significantly higher ($P < 0.05$) than in PW-acclimated controls (Table 3), but was the same as in the 10% SW bathing medium (~2.7 mmol l^{-1} SO_4).

When *T. texasensis* was transferred from 10% SW into

Table 3

Concentration of solutes in the blood of Toxolasma texasensis *acclimated to pondwater (PW), 10% seawater (SW), or returned to PW for various periods*

	mosm kg^{-1}	Ion concentration, mmol l^{-1}					
Treatment	Total solute	Na	K	Ca	Mg	Cl	SO_4
10% SW	110 ± 1f	45.1 ± 0.7e	1.6 ± 0.1e	2.1 ± 0.2a	3.3 ± 0.3e	41.1 ± 1.7e	2.8 ± 0.2c
1 h PW	102 ± 2e	41.0 ± 0.7d	1.1 ± 0.0d	2.8 ± 0.2b	3.6 ± 0.2e	36.2 ± 1.1d	2.1 ± 0.4ab
4 h PW	79 ± 1d	29.6 ± 0.7c	0.9 ± 0.0cd	2.1 ± 0.1a	2.7 ± 0.2d	24.9 ± 0.8c	2.4 ± 0.1bc
8 h PW	67 ± 1c	27.7 ± 0.5c	0.7 ± 0.1bc	2.6 ± 0.1b	1.8 ± 0.2c	21.0 ± 0.3b	1.4 ± 0.2a
24 h PW	55 ± 1b	20.1 ± 0.7b	0.6 ± 0.0ab	3.0 ± 0.2bc	1.3 ± 0.1bc	15.5 ± 0.6a	1.4 ± 0.2a
48 h PW	45 ± 3a	16.4 ± 1.1a	0.4 ± 0.0a	2.6 ± 0.1b	1.0 ± 0.1b	12.0 ± 0.8a	1.4 ± 0.2a
72 h PW	46 ± 1a	16.6 ± 0.2a	0.6 ± 0.1ab	2.9 ± 0.2b	0.8 ± 0.1ab	12.3 ± 0.5a	2.1 ± 0.1abc
PW	44 ± 2a	19.1 ± 0.9b	0.5 ± 0.0a	3.5 ± 0.2c	0.4 ± 0.0a	12.7 ± 0.7a	1.7 ± 0.2a

Data are expressed as mean ±1 standard error, with 5–11 animals for each treatment. Values within a column that have different letters are significantly different using Fisher's protected least significant difference method ($P < 0.05$).

PW, PEG and $^{35}SO_4$ clearance increased (Table 2). Although there was an immediate rise in osmotic uptake of water, only PEG clearance increased significantly during the first hour relative to 10% SW animals, but clearance of both solutes was elevated by 4 h. The clearance of PEG was restored to the same level as that found in PW mussels by 72 h. In contrast, the $^{35}SO_4$ clearance remained elevated for 8 h and then returned to PW control levels by 24 h. The elevation in SO_4 excretion was due to a significant increase in filtration. During the first hour after transfer to PW, the SO_4 clearance remained statistically the same as the PEG clearance. By 4 h, the SO_4 clearance was significantly less ($P < 0.05$) than the corresponding PEG clearance. The reduction in SO_4 clearance was due to the rapid restoration of sulfate reabsorption (Table 2). During the first hour of re-acclimation to PW, the sulfate concentration in the blood returned to the same level as in the PW-acclimated controls; recovery was due to dilution caused by the osmotic uptake of water combined with increased levels of filtration (Table 3). However, 48 h were required for the total solute and most of the other measured ions to return to PW levels (Table 3).

To determine whether the renal tissue of *T. texasensis* could secrete organic acids, radioactive PEG and PAH were both injected into PW-acclimated animals. The clearance was 1.26 ± 0.08 ml g^{-1} dry tissue h^{-1} for PEG, and 5.75 ± 0.65 ml g^{-1} dry tissue h^{-1} for PAH ($n = 10$). PAH is a smaller molecule than PEG, but the volume of blood cleared of PAH by filtration was likely to be the same as for PEG. The additional PAH clearance was due to secretory mechanisms and amounted to 4.49 ml g^{-1} dry tissue h^{-1}; this value was 3.5 times the amount of PAH cleared by filtration.

Discussion

Toxolasma texasensis has a tubular kidney with functional characteristics similar to those found in other invertebrates and vertebrates. The kidney forms urine by ultrafiltration, for which PEG serves as a useful marker (Hevert, 1984). Some solutes can be added to the urine by the process of secretion, as well as filtration, and PAH is an organic acid that is subject to secretory activity. Most of the PAH eliminated by the kidney of *T. texasensis* was through secretory mechanisms. The importance of secretion in the elimination of PAH has also been documented in the snail *Achatina fulica* (Martin *et al.*, 1965). At low concentrations of PAH in the blood, most of this solute is secreted by the snail kidney rather than filtered. The third major process responsible for urine formation is solute reabsorption. In this study, we have focused on the characteristics of sulfate reabsorption by the kidney of *T. texasensis*.

Toxolasma texasensis was able to maintain a sulfate concentration in the blood of about 1–2 mmol l^{-1} while acclimated to an artificial PW containing 0.2 mmol l^{-1} SO_4. Sulfate balance was maintained by transport systems in the epithelia, including the kidney. Sulfate concentrations in the blood and pericardial fluid are the same, which suggests that the anion is freely filtered in molluscs (Potts and Todd, 1965) as it is in vertebrates (Mudge *et al.*, 1973). Thus, filtration in bivalves was assumed to be the same for sulfate as for PEG, but the renal reabsorption of SO_4 reduced its clearance from the blood by more than 80% relative to PEG clearance. Renal reabsorption of sulfate was reduced in animals that were acclimated to 10% SW for almost 2 weeks. During acclimation to 10% SW, the concentration of SO_4 in the blood increased to about 2.8 mmol l^{-1} (equal to the bathing medium). These data contrast with the somewhat more rapid SO_4 transport rates observed in *Dreissena polymorpha*, and with the apparent cessation of SO_4 reabsorption in that species when acclimated to 10% SW (Dietz and Byrne, 1999). The low blood SO_4 concentration of 0.7 mmol l^{-1} reported for the unionid *Anodonta cygnea* (Potts, 1954a) is similar to concentrations we observed in *T. texasensis*, but is less than half the concentration found in *D. polymorpha* (Dietz and Byrne, 1999).

In previous studies, the clearance values for PEG, inulin, and high-molecular weight dextran from the blood of *D. polymorpha* were similar, and we concluded that these three solutes were probably measuring the renal filtration rate (Dietz and Byrne, 1997, 1999). PEG clearance values in PW-acclimated *T. texasensis* were about 1 ml g^{-1} dry tissue h^{-1}, and were similar to inulin clearances reported for the freshwater snail *Lymnaea stagnalis* (de With and van der Schors, 1984). When *T. texasensis* was acclimated to 10% SW, the PEG clearance decreased to about 0.2 ml g^{-1} dry tissue h^{-1}. Although pondwater-acclimated *D. polymorpha* clear the blood of PEG at about double the rate observed for *T. texasensis*, the response by the kidney was the same in both species when the osmotic gradient was abolished by acclimation to 10% SW (Dietz and Byrne, 1997, 1999; this study).

In the sulfate-rich 10% SW environment, *T. texasensis* became isosmotic and isoionic for SO_4 and reduced its renal reabsorption. All freshwater bivalves studied become isosmotic when exposed to dilute seawater (Wilcox and Dietz, 1998; Jordan and Deaton, 1999). They have limited tolerance, but may survive in an environment in which total solutes approach 400 mosm kg^{-1}. Freshwater bivalves can maintain cellular volume regulation under moderate osmotic challenges; their ability to mobilize free amino acids is restricted, however, and this restriction may be responsible for the limit to their survival (Dietz *et al.*, 1998; Jordan and Deaton, 1999).

Transferring mussels from 10% SW to PW would increase the osmotic uptake of water and the subsequent excretion of water by the kidney. After the transfer, PEG clearance was rapidly elevated to values exceeding those observed for PW-acclimated *T. texasensis*. Because of ele-

vated filtration, it was 24 h before SO_4 clearance returned to PW control levels, even though SO_4 reabsorption was immediately reestablished. *D. polymorpha* also required about 24 h to reestablish SO_4 reabsorption to PW control levels (Dietz and Byrne, 1999). However, *D. polymorpha* did not elevate PEG clearance above that found in PW-acclimated animals. Unlike the unionids, *D. polymorpha* has maximum renal filtration (PEG clearance) when acclimated to PW, and filtration cannot be increased even when the mussel is subjected to higher osmotic uptake of water (Dietz and Byrne, 1999).

Recent studies have examined mechanisms of sulfate transport in a variety of preparations (Larsen and Simonsen, 1988; Cole and Rastogi, 1991; Tenenhouse and Martel, 1993; Grassl, 1996; Dietz and Byrne, 1999). Toad skin is capable of active sulfate influx from a Ringer's solution containing 1 mmol l^{-1} SO_4, using an anion exchange mechanism (Larsen and Simonsen, 1988). The anion transport inhibitor, DIDS, significantly decreased the amount of $^{35}SO_4$ label that accumulated in the blood of freshwater bivalves, suggesting that sulfate uptake was linked to an anion exchange mechanism (Dietz and Byrne, 1999; this study).

Sulfate reabsorption is subject to regulatory mechanisms in freshwater bivalves. When mussels were acclimated to 10% SW, the concentration of SO_4 in the blood rose, and renal reabsorption was reduced in both unionids and dreissenids; thus, conservation of this anion was minimal (Dietz and Byrne, 1999; this study). Freshwater bivalves that are acclimated to PW are in a low SO_4 environment, but transport systems and conservation mechanisms both allow them to maintain a SO_4 concentration almost 10 times higher than that in the medium.

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